AKT2 Positive Control
(Human, recombinant protein expressed in Sf9)
Cat# CY-E1168-2

Lot No.
For 200 Assays
(6.25 mUnits / µL x 800 µL)

10X BSA (100 µg/mL x 0.25 mL) is supplied to make an enzyme dilution buffer.

Product Description:
Active human AKT2 containing a *N*-terminal GST-tag and a C-terminal His-tag was produced by co-infection of PDK-1 and full length AKT2 expressing recombinant baculovirus into sf9 cells. Purified by using GSH agarose chromatography. The AKT2 Positive control is designed to use for CycLex AKT Assay/ Inhibitor Screening Kit (Cat# CY-1168). The AKT2 Positive Control should be added to the well at 25 m units/well. For instance, diluted positive control 1:2.5, use 10 µL for 1 assay. Unused AKT2 Positive control should be stored at -70°C.

Product Size: Recombinant AKT2: 5 units/800 µL

Formulation: The AKT2 Positive Control is supplied frozen in a buffer containing 20mM Hepes-KOH (pH 7.5), 1 % BSA, 1mM EDTA, 1 mM DTT, 50mM NaCl, 0.03 % Brij35 and 50% glycerol.

Dilution: The AKT2 Positive control should be diluted with an enzyme dilution buffer to avoid inactivating the enzyme activity in low protein concentration condition. Enzyme dilution buffer: Mix 9-parts of Kinase buffer and 1-part of 10X BSA (100 µg/mL x 0.25 mL)

Source: Human AKT2 containing N-terminal GST-tag and C-terminal His-tag, expressed in sf9 cells.

Molecular Weight: AKT2 Positive Control demonstrates a doublet 92 kDa bands by SDS-PAGE analysis.

Purity: AKT2 Positive Control is greater than 90% pure as determined by SDS-PAGE analysis.

Substrates: AKT2 phosphorylates a number of substrates, including GSK-3alpha, Apaf-1, hTERT, Bad and MBP.

Inhibitors: AKT2 specific inhibitor has not been discovered yet.

Unit Definition: One unit is defined as the amount of kinase required to incorporate 1nmol of phosphate into the GST-Bad per minute at 30°C.

Assay Conditions: Assay activity of AKT2 in a 50 µL reaction containing 20 mM Hepes KOH (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, 100 µM [gamma ^{32}P] ATP (1 µCi), and 4 µg of GST-Bad fusion protein. Start the reaction by adding 10µL of the enzyme, diluted 50-fold in a buffer containing 20 mM Hepes KOH (pH 7.5), 1 mM DTT, 0.03 % Brij35. Incubate for 30 minutes at 30°C. Terminate the reaction by adding 600 µL of cold 10 % TCA solution containing 0.2 % Sodium pyrophosphate and stand on ice for 15 min. Filtrate acid insoluble material through GFC filters (Whattman Inc.), wash 4

times with 1 % TCA and rinse filters with ethanol. Dry filters and count in a liquid scintillation counter.

Storage and Stability: Stable for 12 months at -70°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot enzyme to avoid repeated freezing and thawing.

Related Products:
* AKT Assay/Inhibitor Screening Kit: Cat# CY-1168
* AKT1 Positive Control: Cat# CY-E1168-1

References:
1. Staal, S. P. *Proc. Nat. Acad. Sci*. **84**, 5034-5037, 1987.
2. Andjelkovic M, Alessi DR, Meier R, Fernandez A, Lamb NJ, Frech M, Cron P, Cohen P, Lucocq JM, Hemmings BA. *J Biol Chem.* **272**, 31515-24, 1997
3. Meier, R., Alessi, D. R., Cron, P., Andjelkovic, M. and Hemmings, B. A. *J. Biol. Chem.* **272**, 30491–30497, 1997
4. Cross, D. A.E., Alessi, D. R., Cohen, P., Andjelkovic, M., and Hemmings, B. A. *Nature* **378**, 785-789, 1995
5. Kohn, A. D., Summers, S. A., Birnbaum, M. J., and Roth, R. A. *J. Biol. Chem.* **271**, 31372-31378, 1996
6. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. *Science* **275**, 661-665, 1997
7. Cheng, J.Q., Godwin, A. K., Bellacosa, A., Taguchi, T., Franke, T. F., Hamilton, T. C., Tsichlis, P. N., and Testa, J. R. *Proc. Nat. Acad. Sci*. **89**, 9267-9271, 1992
8. Bellacosa, A.et al. *Int. J. Cancer* **64**, 280-285, 1995
9. Cheng, J.Q., Ruggeri, B., Klein, W. M., Sonoda, G., Altomare, D. A., Watson, D. K., and Testa, J. R. *Proc. Nat. Acad. Sci.* **93**, 3636-3641, 1996
10. Vanhaesebroeck, B.; Alessi, D. R. *Biochem. J.* **346**, 561-576, 2000. (Review)
11. Franke, T. F., Kaplan, D. R., and Cantley, L. C. *Cell* **88**, 435-437, 1997 (Review)
12. Hemmings, B. A. *Science* **275**, 628-630, 1997 (Review)

PRODUCED BY

CycLex Co., Ltd.
1063-103 Terasawaoka
Ina, Nagano 396-0002
Japan
Fax: +81-265-76-7618
e-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp